European Journal of Clinical Investigation (1997) **27**, 802–811

REVIEW

Metabolic and genetic aspects of familial combined hyperlipidaemia with emphasis on low-density lipoprotein heterogeneity

S. J. H. BREDIE, P. N. M. DEMACKER & A. F. H. STALENHOEF Department of Medicine, Division of General Internal Medicine, University Hospital Nijmegen, Nijmegen, The Netherlands

Received 25 February 1997; accepted 7 June 1997

Introduction

The relationship between elevated plasma cholesterol and the risk of coronary artery disease is now definitively established [1–5]. Accumulating evidence indicates that the total amount of triglyceride-rich lipoprotein particles, i.e. chylomicron remnants, very low-density lipoproteins (VLDLs) and intermediate-density lipoproteins (IDLs), also determines the risk of developing cardiovascular disease [6–9]. This would explain the benefit of cholesterol-lowering therapy observed in the majority of patients with coronary disease who have only marginally elevated plasma cholesterol levels but may exhibit other lipid abnormalities [4].

In patients suffering from familial combined hyperlipidaemia (FCH), elevated levels of triglyceride-rich lipoproteins mainly determine the presenting lipid phenotype. Because FCH appears to be the most common form of hyperlipidaemia in young survivors of myocardial infarction [10–12], causing an estimated 10% of all premature coronary heart disease [13,14], recent research has been focused on the pathophysiological mechanism underlying premature atherogenesis in FCH. In this review, hypotheses concerning the metabolic and genetic basis of FCH and its related entities, as well as the origin of LDL heterogeneity associated with these lipid disorders, will be discussed.

Phenotypic diagnosis of FCH

In 1973, FCH was first described by Goldstein *et al.* [10], Rose *et al.* [13] and Nikkila and Aro [11], shortly followed by others [15,16], as a new autosomal dominant inherited lipid disorder characterized by elevated plasma cholesterol and triglyceride levels in first-degree relatives and strongly associated with premature cardiovascular disease. At that time, the recognition of FCH confounded the previously formulated Fredrickson classification of hyperlipoproteinaemias by the presence of first-degree relatives exhibiting different lipid phenotypes within one single family.

Because there is no specific marker for the disorder whereas the lipid phenotypic expression among affected individuals may show some variation with time, the diagnosis is necessarily based on family investigation to demonstrate a so-called 'mixed hyperlipidaemia' with either hypercholesterolaemia, hypertriglyceridaemia or combined hyperlipidaemia in first-degree relatives. Criteria supporting the FCH diagnosis are presented in Table 1. Nowadays, it is common sense that all main inclusion criteria, in the absence of all exclusion criteria, should be met for a true diagnosis, whereas the diagnostic value of the mentioned additional inclusion criteria is still under debate.

Although FCH patients frequently exhibit elevated plasma apo B concentrations [14,17] when compared with their normolipidaemic relatives, the interpretation of total plasma apolipoprotein B (apo B) levels as a diagnostic criterion is still open for discussion. A plasma apo B100 level above 130 mg dL^{-1}, standardized according to the radioimmunoassay method of the International Union of Immunological Societies, may contribute to defining FCH patients [18,19]. Considering that the lipid to protein ratio of VLDL and LDL particles is relatively constant even in FCH patients [14,20,21], total plasma apo B could be derived from the strong correlation that appears to exist between VLDL- plus LDL-cholesterol and plasma apo B [17]. Therefore, plasma apo B is strongly correlated with the presented FCH lipid phenotype based on elevated VLDL and/or LDL concentrations [17].

For reasons that are not clear, the manifestation of hyperlipidaemia in childhood, as frequently observed in familial hypercholesterolaemia, rarely occurs in FCH [22]. However, hyperapobetalipoproteinaemia, a feature associated with FCH, has been detected in children of parents with premature cardiovascular disease [23,24].

Correspondence: A. F. H. Stalenhoef, Department of Medicine, Division of General Internal Medicine, 541, University Hospital Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands.

Table 1. Inclusion and exclusion criteria supporting the diagnosis familial combined hyperlipidaemia

Main inclusion criteria
Presence of a multiple type hyperlipidaemia in first-degree relatives of a single family comprising hypertriglyceridaemia, hypercholesterolaemia, and combined hyperlipidaemia, as defined by fasting plasma cholesterol and/or plasma triglyceride concentrations above the 90th percentile for age and gender
Autosomal dominant inheritance of the hyperlipidaemia
Presence of premature atherosclerosis (before age of 60 years) in first-degree relatives
Additional inclusion criteria
An elevated total plasma apolipoprotein-B concentration
A LDL subfraction profile predominated by small, dense LDL particles
Manifestation of the hyperlipidaemia in adolescence
Exclusion criteria
Presence of any form of xanthoma in first-degree relatives
Presence of a secondary cause for the hyperlipidaemia in affected relatives
Presence of the Apo ε2/ε2 genotype in first-degree relatives

FCH and its related lipid phenotypes

Initially FCH was thought to be inherited as a single-gene disorder with a major effect on triglyceride levels [10]. Recently, evidence for a major gene effect on triglyceride levels was again provided by complex segregation analysis in British FCH families [25]. Although this supposed gene mutation has still not been located, other studies have indicated that a variety of metabolic and biochemical defects predispose for the FCH phenotype, suggesting that the genetic basis of this trait is heterogeneous and may even involve several defects in one family. According to these reports FCH may be considered more as a 'syndrome', showing overlapping characteristics with other entities (Fig. 1) such as (a) hyperapobetalipoproteinaemia (hyperapoB) defined by a normal LDL-cholesterol level with an increased LDL protein (apo B) content [26]; (b) the 'atherogenic lipoprotein phenotype' (ALP) characterized by increased triglyceride and apo B levels, decreased HDL levels and a predominance of small, dense LDL [27]; (c) familial dyslipidaemic hypertension (FDH), a syndrome of mixed lipid abnormalities resembling the FCH phenotype, associated with mild hypertension [28]; and (d) the insulin resistance syndrome, which is associated with increased VLDL production and impaired clearance of triglyceride-rich particles, also key features of FCH [29,30].

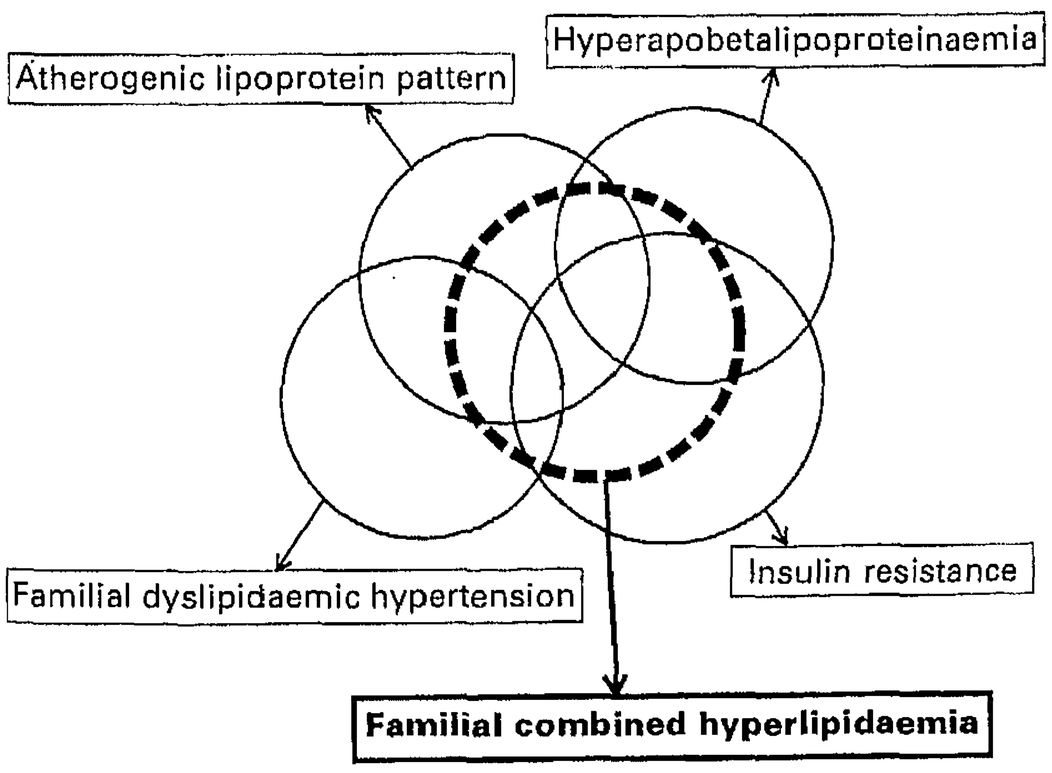

Figure 1. Overlapping characteristics of four entities with key features of the FCH phenotype, which may contribute to the complete picture of FCH (adapted from Kwiterovich POJ, Curr. Opin Lipidol 1993;4:133–43).

Pathophysiology of FCH

In general, FCH is thought to be caused by hepatic overproduction of VLDL with or without impaired clearance of triglyceride-rich lipoproteins [31,32]. As no single metabolic defect detected so far can fully account for the FCH phenotype, it has been hypothesized that a number of defects are involved. It still has to be determined whether these defects are causal of the disorder or are a regulatory consequence of an underlying metabolic defect. In general, circulating triglyceride-rich lipoproteins are of exogenous or endogenous origin. For better comprehension, these pathways are described in more detail below.

The exogenous pathway

This pathway involves the transport of dietary lipids from the intestine to the liver by apo B48-containing chylomicrons. As a result of the action of the enzyme lipoprotein lipase (LpL), activated by co-factor apo CII, fatty acids are liberated from chylomicrons and pass to the adipose tissue or skeletal muscle cells to be oxidized or stored. Reduced LpL activity as a result of *LpL* gene mutations has been reported repeatedly in subsets of FCH populations [33,34] and may result in impaired clearance of chylomicrons. For storage in adipocytes, free fatty acids (FFAs) are intracellularly re-esterified to triglycerides, a process that is mediated by the action of a basic protein called acylation stimulatory protein (ASP) [35]. Owing to impaired ASP activity, as reported in hyperapoB, a reduced rate of FFA uptake into adipocytes may result in an increased flux of FFA to the liver and

consequently in increased hepatic VLDL synthesis [36]. After the release of FFAs, the remaining chylomicron remnant particles are taken up by the liver via a specific remnant receptor that only recognizes apo E as ligand [37]. A delayed clearance of atherogenic chylomicron remnants, possibly due to competition between chylomicrons and endogenous VLDL for available LpL activity and competition for remnant receptor capacity, has been reported to exist in FCH patients [38]. In the hepatocytes, all components of the remnants are hydrolysed in the lysosomal compartment and a part of this material is re-used to form nascent VLDL particles entering the endogenous pathway. Intracellular increase in cholesterol in hepatocytes may increase plasma LDL-cholesterol concentration as a result of hepatic LDL receptor down-regulation.

The endogenous pathway

This pathway involves the assembly of formed endogenous cholesterol and triglycerides in the core of VLDL followed by excretion into the circulation. *In vitro* studies show that in HepG2 cells intracellular triglyceride biosynthesis, but not the rate of synthesis of cholesterol or cholesteryl esters, determines the secretion rate of VLDL-apo B [39]. The triglyceride biosynthesis itself depends on the availability of required FFAs, which are either released from adipocytes, stored intrahepatically or converted from dietary carbohydrates [40]. The release of required FFAs from visceral adipocytes is mediated by the action of the enzyme hormone-sensitive lipase (HSL) [41,42]. Post-prandial hyperinsulinaemia plays a regulatory role because it inhibits the lipolytic effect of HSL to allow FFA uptake by adipocytes [43]. FCH is associated with increased insulin resistance, which would allow for increase in VLDL production by net increase of serum FFAs [44,45]. Without merging with lipids to allow the formation of a VLDL particle, nascent apoprotein B100 is degraded. This process is catalysed by the action of microsomal triglyceride transfer protein (MTP), referring to its site of action in the hepatic endoplasmic reticulum [46,47]. Recently, abetalipoproteinaemia, the metabolic 'opposite' of FCH, was found to be caused by MTP absence [48]. Consequently, it has to be established whether MTP overexpression could also play a role in VLDL-apo B overproduction of FCH.

The continuous hydrolysis of core triglycerides in FFAs by LpL converts VLDLs into smaller apoprotein B100-containing VLDL remnants, IDL and LDL [40,49,50]. Recent reports suggest that a heterozygous state for one of the mutations found in the *LpL* gene, affecting its activity, may result in a lipoprotein pattern classified as FCH [33,34,51]. However, it remains unclear whether this phenomenon is more pronounced in hyperlipidaemic subjects than in normolipidaemic individuals without an underlying metabolic defect. Accurate data on the prevalence of these mutations in different populations may help to interpret the observed influences. Increased apo CIII levels are associated with impaired clearance of triglyceride-rich lipoprotein due to direct inhibition of LpL by apo CIII [52]. Interestingly, linkage between the FCH phenotype and the *AI/CIII/AIV* gene cluster has been reported [53]. However, this finding could not be confirmed by others, although several polymorphisms in the gene cluster were recently found to amplify the phenotypic expression in FCH [54].

A portion of small VLDL, i.e. IDL, is catabolized after apoprotein E-mediated binding to hepatic LDL or B/E receptors, which differ from the chylomicron remnant receptor. Affinity for the B/E receptor depends on the apo E isoform (i.e. high for apo E3 and E4, but low for apo E2). A recent study on the effects of apo E polymorphism on presenting lipid phenotype in FCH suggested that differences in apo E isoform-related clearance may only contribute to the hyperlipidaemia as a result of other defects [55]. Further hydrolysis of triglycerides, predominantly by hepatic triglyceride lipoprotein lipase (HtgL), processes remaining IDL into LDL particles that then mainly consist of cholesteryl esters and apoprotein B100 [56]. Exchange of LDL cholesteryl esters with VLDL triglycerides mediated by cholesteryl ester transfer protein (CETP) activity determines in part the observed heterogeneity of LDL particles [57], as will be discussed later.

FCH and LDL heterogeneity

Introduction

It has been recognized for a number of years that LDL particles are markedly heterogeneous in physical and chemical properties [58–62]. In FCH, these properties of LDL are reported to be different from normal [20,63,64]. The LDL subspecies in FCH are heterogeneous with a propensity towards small, dense particles [14,20,65]. The predominance of small, dense LDL subfractions in FCH family members may not be fully explained by metabolic processing alone. Direct, as yet unclarified, genetic influences on the distribution are proposed as well [17,66,67]. Also, it is only recently that the relationship between qualitative features of LDL particles and cardiovascular disease has attracted considerable interest. This interest was raised by reports that certain LDL classes may be more atherogenic than others owing to differences in susceptibility towards oxidative modification [59,68–71]. Recent prospective studies supported this evidence of a role for small, dense LDL particles in the aetiology of atherosclerosis [72–74]. However, major questions about origin, structural variation and biological function of LDL subspecies are still only partly understood.

Identification and characterization of LDL subfractions

Since the first reports on measurements of LDL heterogeneity, two basically different techniques have been used to identify LDL subspecies: (a) non-denaturing gel electrophoresis (GGE) of whole plasma or of isolated LDL, which separates several LDL subspecies based on

differences in size [58]; and (b) density gradient ultracentrifugation (DCUG) of whole plasma, based on differences in density within the LDL subclass population [75,76]. Nowadays, both techniques are widely used in large-scale studies to identify LDL heterogeneity.

Using GGE, most individuals are characterized by only two or three peaks or shoulders on the densitometric scan. Based on the location of these peaks on the gel, LDL subclass pattern can be classified dichotomously into pattern A, predominantly characterized by large LDL particles, and pattern B, in which small LDL particles predominate [27,69]. Recently, the LDL peak particle diameter was defined by the estimated diameter or size of the major observed LDL subclass, to classify LDL subfraction pattern in a more continuous fashion [77]. Single-spin DGUC procedures are designed for optimal resolution of prestained apo B-containing lipoproteins [60,62,64,78,79]. Depending on the salt gradient used and ultracentrifugation performed, up to 15 fractions can be isolated within the LDL density of 1·019–1·063 g mL^{-1}. A single-spin DGUC procedure using prestained whole plasma has been the basis of the research in our laboratory. This method reveals up to five different LDL subfractions. Quantification by densitometric scanning allows the calculation of a continuous variable K describing the relative contribution of each LDL peak height to the total LDL [80]. In a comparison study, the number of LDL subfractions detected by GGE or DGUC was the same for more than 90% of the sera [81]. However, different LDL subfractions were less well separated by GGE than by DGUC. Furthermore, an advantage of the DGUC is that isolated samples of subclasses are available for further biochemical analysis. In the near future, a capillary electrophoretic technique may combine the advantages of a fast procedure and small amount of required plasma to discriminate between different LDL subfractions [82].

Metabolic aspects of LDL subfractions

The intravascular formation of LDL subfractions involves the conversion of VLDL precursors [83,84], and possibly also a direct hepatic secretion of different IDL [85] or LDL subspecies [31,86]. Previously, it was postulated that exchange of LDL cholesteryl ester for VLDL triglyceride, mediated by the CETP, results in a net transfer and a significant enrichment of the LDL with triglyceride [87–89]. The subsequent action of lipoprotein lipase (LpL) or hepatic lipase (HtgL) results in hydrolysis of a significant amount of LDL triglycerides and thereby a decrease in particle size [90–93]. The rate and magnitude of exchange may depend upon the relative pool size of triglyceride-rich lipoproteins vs. the cholesteryl ester-rich lipoproteins. In general, this hypothesis of exaggerated triglyceride transfer and lipolysis can explain the predominance of small, dense LDL in any form of hypertriglyceridaemia, but in FCH the pool of triglyceride-rich lipoproteins is primarily enlarged and consists of chylomicron remnants as well [38]. However, few data, including our own, also support the presence of a predominance of dense particles in FCH family members who display primarily elevated cholesterol levels [66,67,94]. Furthermore, VLDL heterogeneity may also underlie LDL heterogeneity. Large triglyceride-rich VLDL, resembling chylomicrons in patients with LpL deficiency, were found to be rapidly removed from the circulation [50]. Several studies demonstrated that predominantly small VLDL particles secreted into the circulation are converted into LDL [49,95]. Using stable isotopes, it was recently demonstrated that, in subjects with predominantly dense LDL, both an increased production and reduced clearance of large VLDL occurred, which then undergo intravascular catabolism to successively smaller remnant particles, a pathway not apparent in subjects with larger, more buoyant LDL [96]. This and numerous other studies demonstrate the complexities of apo B particle metabolism. However, all these studies have in common that the metabolic actions of lipid transfer proteins and lipases, eventually combined with substrate specificity as well as heterogeneity among apo B precursor particles, could account for the multiple different LDL subfractions observed in normal and hyperlipidaemic subjects.

Genetic aspects of LDL heterogeneity

Accumulating data suggest that the formation of LDL subfraction profiles is influenced genetically [97]. In particular, the finding of inherited LDL subfraction profiles in normolipidaemic families [80,98] strongly suggests a genetic background. Initially, Fisher *et al.* [99] reported a single genetic locus without dominance thought to be responsible for inheritance of LDL molecular weight quality in five families. Complex segregation analysis in healthy families [98] and in families of probands with familial combined hyperlipidaemia [66] have indicated that LDL subclass pattern B, as assessed by gradient gel electrophoresis, is under the influence of a major gene or genes with a dominant or additive mode of inheritance. Two recent studies, a study in normolipidaemic families [80] and a study in FCH families [67] from our laboratory, in which LDL subfractions were detected by DCUG, confirmed a major gene effect. In contrast to the previous studies of Austin *et al.* [66,98], we observed a recessive mode of inheritance and gene frequencies significantly different between the normolipidaemic and FCH population [67,80]. However, all these studies, including a recently performed heritability analysis of a continuous LDL peak particle diameter performed in twins [100], have indicated that genetic factors could account for at least 40% of the variation in LDL particle size and density in both normolipidaemic and hyperlipidaemic subjects, with the remaining 60% due to non-genetic or environmental influences. Among these environmental factors are age, gender, body mass index, smoking habits, hormonal status in women (combined estimated effect of 20%) and lipid and lipoprotein levels (estimated effect of 40%) [67,80].

Additional genetic studies have linked candidate genes to the small, dense LDL phenotype. Although reported

only once, remarkably strong linkage (LOD score of 4·43) of pattern B to a gene locus near the LDL receptor on chromosome 19p was found in 51 family members of nine probands with an 'atherogenic lipoprotein phenotype' (ALP), whereas weaker linkage was observed with the insulin receptor locus on the same chromosome 19p [101]. Recently, in subjects of the San Antonio Heart Study, variations in apo E phenotype were associated not only with changes in the absolute LDL-cholesterol concentration, but also with changes in its composition [102]. However, in 201 affected FCH subjects of our families, these influences of apo E polymorphism on LDL heterogeneity were not observed [55]. In another study, the apo B100 *Eco*RI polymorphism, previously associated with variation in plasma lipids, was found to play a role in the susceptibility to the development of dense LDL in viscerally obese hyperinsulinaemic men [103]. Evidence for linkage of pattern B to three markers on the LDL receptor itself – the *apo CIII* gene on chromosome 11, the *CETP* gene on chromosome 16p and the manganese superoxide dismutase (*MnSOD*) gene on chromosome 6q – has also been reported. However, no linkage was observed for other candidate loci tested: apo B, apo AI, apo (a), apo E/CI/CIII, LpL and HDL-binding protein [104,105]. Although the investigated genetic loci have been identified by polymorphic DNA markers, which do not necessarily indicate the presence of causative mutations, it is remarkable that the protein products of genes with observed linkage have connections with metabolic pathways possibly involved in the generation or an impaired clearance of small, dense LDL

1 Because small, dense LDL particles have been shown to have reduced affinity for the LDL receptor [106–108], altered LDL receptor function or regulation could result in further impairment of plasma clearance of these LDL or their metabolic products.
2 *Apo CIII* gene haplotypes are associated with variation in plasma triglyceride levels [52], which in turn could affect levels of small, dense LDL.
3 CETP may facilitate lipolytic conversion of larger to smaller LDL particles by promoting triglyceride transfer into the LDL core [109].
4 A possible mechanism associated with MnSOD activity is unclear, but it is conceivable that defective function of MnSOD results in increased lipid hydroperoxides in plasma lipoproteins, with a concomitant increase in oxidative susceptibility, or otherwise alters lipoproteins in a manner leading to formation of small, dense, more oxidizable LDL [71,105,110].

Although genetically influenced factors resulting in retardation of catabolism of triglyceride-rich lipoproteins or their remnants may have an aetiological or contributory role in the formation of small, dense LDL, it is striking that mutations in the gene coding for LpL were not linked to the pattern B [105] even so, because the LpL Asn291→Ser was recently found to be significantly linked to the presenting lipid phenotype in FCH families [51].

Altogether, LDL heterogeneity results from a variety of environmental influences and probably also from direct genetic factors. In a family, one or more defect may be responsible for the major gene and additive effects identified by segregation analysis.

FCH and premature atherogenesis

In spite of often mildly elevated lipid levels compared with other lipid disorders, a high prevalence of cardiovascular diseases occurs in FCH families. The explanation for premature cardiovascular disease in FCH may be attributed to the increased prevalence of small, dense LDL [27,111].

One of the earliest events in the formation of atherosclerotic plaques is the massive accumulation of cholesterol in so-called scavenger cells to convert into foam cells in the artery wall [112,113]. As normal receptor-mediated endocytosis of cholesterol via the LDL receptor initiates intracellular processes that prevent further LDL uptake, alternative mechanisms are necessary to explain the foregoing intracellular cholesterol accumulation [114]. Many lines of evidence support the hypothesis that oxidative modification of LDL plays a pivotal role in atherogenesis [115–118]. However, this theory cannot be considered proven for the human disease [119]. The oxidative modification hypothesis proposes that oxidative damage to LDL generates a series of modified forms of LDL (oxLDL) that are in a number of ways more atherogenic than native LDL. In contrast to native LDL, oxidized LDL (oxLDL) is recognized and rapidly internalized by macrophage scavenger receptors [115], exhibits marked effects on the viability of endothelial cells and smooth muscle cells and alters the chemotactic activity of monocytes and monocyte-derived macrophages, all features which have been implicated in atherogenesis [115]. Special oxLDL receptors on the macrophages may not be down-regulated by the endocytosis of several forms of modified LDL and facilitate intracellular accumulation of oxLDL [120–123]. Small, dense LDL, being more susceptible to oxidative modification [64,71,124], may increase the supply of oxLDL to these receptors. Recently, in subjects with FCH, total LDL was found to be more prone to *in vitro* oxidation owing to the predominance of dense LDL particles. In addition, it was suggested that the decreased redox status of coenzyme Q10 in LDL from subjects with a dense LDL subfraction profile reflected the presence of already *in vivo* modified LDL owing to lipid peroxidation in the circulation [125,126].

Therapeutic options in FCH

Because of the up to 10-fold increased incidence of cardiovascular diseases in FCH patients [10,14], family screening and lipid-lowering treatment should be initiated. Both lowering of the total amount of atherogenic lipoproteins, i.e. LDL-cholesterol and triglyceride-rich lipoproteins, as well as a reduction in the atherogenicity of LDL, i.e. reduction in the total amount of small, dense

LDL particles, should be aimed at. For this purpose, diet and lifestyle changes are usually insufficient and, consequently, drug therapy is frequently indicated. In the last decade, a spectrum of effective lipid-lowering drugs became available. The HMG-CoA reductase inhibitors are highly effective in reducing LDL-cholesterol in patients with primary hypercholesterolaemia [127]. Although these drugs show some triglyceride reduction as well, less effect is observed in reduction of the amount of small, dense LDL particles [19,128,129]. Fibrates show a primary triglyceride-lowering effect [130]. Convincingly related to this effect on triglyceride levels, a reduction in the size of the small, dense LDL subfraction and normalization of the LDL subfraction profile is observed, whereas the total amount of LDL-cholesterol is unaffected or may even increase [19,131,132]. Nicotinic acid, which especially reduces triglyceride levels by modifying the amount of FFA entering the liver, can be very useful in FCH [3,133,134]. However, because of several side-effects it is prescribed less often in Europe. Bile acid-binding resins are frequently contraindicated in FCH because of an increase in VLDL concentration [135,136]. The place of antioxidants, i.e. vitamins E and C, β-carotene and flavonoids, to prevent LDL particles from oxidative modification and cardiovascular disease is still under investigation. Although a reduction in *in vitro* LDL oxidizability has been observed [137–140] and a reduced risk of coronary heart disease was found [141–146], the results of these studies are not totally consistent.

A direct comparison of the HMG-CoA reductase inhibitor simvastatin and the fibrate gemfibrozil in the treatment of FCH subjects with a combined hyperlipidaemic phenotype demonstrated the specific effect of both drugs. However, none of these agents alone completely normalized the lipid and lipoprotein profiles. Interestingly, an overall dense LDL subfraction profile, although less pronounced, persisted despite substantial triglyceride-lowering [19]. This finding therefore supports the hypothesis of small, dense LDL being present in FCH subjects irrespective of metabolic influences.

So far, the use of drugs should be based on which lipoprotein fraction is elevated the most, and probably a combination of a statin and a fibrate may be the therapy of choice in selected FCH patients with a high risk of cardiovascular disease. In future, possibly more potent HMG-CoA reductase inhibitors, such as atorvastatin, which also have a strong triglyceride-lowering effect, may become the drug of choice [147,148].

Conclusion

Because of its large impact on total cardiovascular mortality, knowledge of the pathogenesis of the heterogeneous FCH syndrome as well as the cause of the associated premature atherogenesis is essential. A major difficulty arises in identifying affected subjects, because a specific marker for the disorder is still lacking. Therefore, family investigation should be performed to verify the diagnosis in a patient with combined hyperlipidaemia and/or premature cardiovascular disease. Until now, this is the only way to prevent affected relatives from premature cardiovascular disease.

References

1 Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet 1994;344:1383–9.

2 Shepherd J, Cobbe SM, Ford I *et al.* Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. N Engl J Med 1995;333:1301–7.

3 Brown G, Albers JJ, Fisher LD *et al.* Regression of coronary artery disease as a result of intensive lipid-lowering therapy in men with high levels of apolipoprotein B. N Engl J Med 1990;323:1289–98.

4 Jukema JW, Bruschke AV, van Boven Aj *et al.* Effects of lipid lowering by pravastatin on progression and regression of coronary artery disease in symptomatic men with normal to moderately elevated serum cholesterol levels. The Regression Growth Evaluation Statin Study (REGRESS). Circulation 1995;91:2528–40.

5 Effect of simvastatin on coronary atheroma: the Multicentre Anti-Atheroma Study (MAAS) [published erratum appears in Lancet 1994 Sep, 10;344[8924]: 762]. Lancet 1994;344:633–8.

6 Austin MA. Plasma triglyceride and coronary heart disease. Arterioscler Thromb 1991;11:2–14.

7 Assmann G, Schulte H, von-Eckardstein A. Hypertriglyceridemia and elevated lipoprotein (a) are risk factors for major coronary events in middle-aged men. Am J Cardiol 1996; 77: 1179–84.

8 Tenkanen L, Pietila K, Manninen V, Manttari M. The triglyceride issue revisited. Findings from the Helsinki Heart Study. Arch Intern Med 1994;154:2714–20.

9 Hokanson JE, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. J Cardiovasc Risk 1996;39:213–19.

10 Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. J Clin Invest 1973;52:1544–68.

11 Nikkila EA, Aro A. Family study of serum lipids and lipoproteins in coronary heart-disease. Lancet 1973;1:954–9.

12 Genest JJ, Martin-Munley SS, McNamara JR *et al.* Familial lipoprotein disorders in patients with premature coronary artery disease. Circulation 1992;85:2025–33.

13 Rose HG, Kranz P, Weinstock M, Juliano J, Haft JI. Inheritance of combined hyperlipoproteinemia: evidence for a new lipoprotein phenotype. Am J Med 1973;54:148–60.

14 Grundy SM, Chait A, Brunzell JD. Familial combined hyperlipidemia workshop. Arteriosclerosis 1987;7:203–7.

15 Brown HB, Lewis LA, Page IH. Mixed hyperlipemia, a sixth type of hyperlipoproteinemia. Atherosclerosis 1973;17:181–96.

16 Natali A, Santoro D, Palombo C, Cerri M, Ghione S, Ferrannini E. Impaired insulin action on skeletal muscle metabolism in essential hypertension. Hypertension 1991;17:170–8.

17 Bredie SJH, van Drongelen J, Kiemeney LA, Demacker PNM, Beaty TH, Stalenhoef AFH. Segregation analysis of plasma apolipoprotein-B levels in familial combined hyperlipidemia. Arterioscler Thromb Vasc Biol 1997;17:834–40.

18 Austin MA, Horowitz H, Wijsman E, Krauss RM, Brunzell J. Bimodality of plasma apolipoprotein B levels in familial combined hyperlipidemia. Atherosclerosis 1992;92:67–77.

19 Bredie SJH, de Bruin TWA, Demacker PNM, Kastelein JJP, Stalenhoef AFH. Comparison of gemfibrozil vs. simvastatin in familial combined hyperlipidemia and effects on apolipoprotein-B-containing lipoproteins, low-density lipoprotein subfraction profile, and low-density lipoprotein oxidizability. Am J Cardiol 1995;75:348–53.

20 Brunzell JD, Albers JJ, Chait A, Grundy SM, Groszek E, McDonald GB. Plasma lipoproteins in familial combined

hyperlipidemia and monogenic familial hypertriglyceridemia. J Lipid Res 1983;24:147–56.
21 Stalenhoef AFH, Demacker PNM, Lutterman JA, van 't Laar A. Plasma lipoproteins, apolipoproteins, and triglyceride metabolism in familial hypertriglyceridemia. Arteriosclerosis 1986;6:387–94.
22 Kwiterovich POJ, White S, Forte T. Bachorik PS, Smith H, Sniderman A. Hyperapobetalipoproteinemia in a kindred with familial combined hyperlipidemia and familial hypercholesterolemia. Arteriosclerosis 1987;7:211–25.
23 Kwiterovich P, Beaty T, Bachorik P, Chen J, Franklin F, Georgopoulos L, Sniderman A. Pediatric hyperlipoproteinemia: the phenotypic expression of hyperapobetalipoproteinemia in young probands and their parents. Prog Clin Biol Res 1988;255:89–105.
24 Sniderman A, Teng B, Genest J, Cianflone K, Wacholder S, Kwiterovich PJ. Familial aggregation and early expression of hyperapobetalipoproteinemia. Am J Cardiol 1985;55:291–5.
25 Cullen P, Farren B, Scott J, Farrall M. Complex segregation analysis provides evidence for a major gene acting on serum triglyceride levels in 55 British families with familial combined hyperlipidemia. Arterioscler Thromb 1994;14:1233–49.
26 Sniderman A, Shapiro S, Marpole D, Skinner B, Teng B, Kwiterovich POJ. Association of coronary atherosclerosis with hyperapobetalipoproteinemia [increased protein but normal cholesterol levels in human plasma low density (beta) lipoproteins]. Proc Natl Acad Sci USA 1980;77:604–8.
27 Austin MA, King MC, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. Circulation 1990;82:495–506.
28 Williams RR, Hunt SC, Hopkins PN *et al.* Familial dyslipidemic hypertension. Evidence from 58 Utah families for a syndrome present in approximately 12% of patients with essential hypertension. JAMA 1988;259:3579–86.
29 Hunt SC, Wu LL, Hopkins PN, Stults BM, Kuida H, Ramirez ME, Lalouel JM, Williams RR. Apolipoprotein, low density lipoprotein subfraction, and insulin associations with familial combined hyperlipidemia. Study of Utah patients with familial dyslipidemic hypertension. Arteriosclerosis 1989;9:335–44.
30 Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 1988;37:1595–1607.
31 Kissebah AH, Alfarsi S, Evans DJ. Low density lipoprotein metabolism in familial combined hyperlipidemia. Mechanism of the multiple lipoprotein phenotypic expression. Arteriosclerosis 1984;4:614–24.
32 Chait A, Albers JJ, Brunzell JD. Very low density lipoprotein overproduction in genetic forms of hypertriglyceridaemia. Eur J Clin Inv 1980;10:17–22.
33 Babirak SP, Brown BG, Brunzell JD. Familial combined hyperlipidemia and abnormal lipoprotein lipase. Arterioscler Thromb 1992;12:1176–83.
34 Reymer PWA, Groenemeyer BE, Gagne E, Miao L, Appelman EEG, Seidel JC, Kromhout D *et al.* A frequently occuring mutation in the lipoprotein lipase gene (Asn291Ser) contributes to the expression of familial combined hyperlipidemia. Hum Mol Genet 1995;4:1543–9.
35 Cianflone KM, Sniderman AD, Walsh MJ, Vu HT, Gagnon J, Rodriguez MA. Purification and characterization of acylation stimulating protein. J Biol Chem 1989;264:426–30.
36 Sniderman AD, Cianflone KM. Substrate delivery as a determinant of hepatic apoB secretion. Arterioscler Thromb 1993;13:629–36.
37 Beisiegel U, Weber W, Ihrke G, Herz J, Stanley KK. The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. Nature 1989;341:162–4.
38 Cabezas MC, de Bruin TW, Jansen H, Kock LA, Kortlandt W, Erkelens DW. Impaired chylomicron remnant clearance in familial combined hyperlipidemia. Arterioscler Thromb 1993;13:804–14.
39 Benoist F, Grand-Perret T. ApoB-100 secretion by hepG2 cells is regulated by the rate of triglyceride biosynthesis but not by intracellular lipid pools. Arterioscler Thromb Vasc Biol 1996;16:1229–35.
40 Havel RJ, Goldstein JL, Brown MS. Lipoproteins and lipid transport. In: Metabolic control and disease, Bondy PK, Rosenberg LE, eds. Philadelphia: Saunders, 1980: 393–494.
41 Tornqvist H, Krabisch L, Belfrage P. Rapid assay for hormone-sensitive lipase activity of adipose tissue. J Lipid Res 1972;13:424–6.
42 Fredrikson G, Stralfors P, Nilsson NO, Belfrage P. Hormone-sensitive lipase of rat adipose tissue. Purification and some properties. J Biol Chem 1981;256:6311–20.
43 Stralfors P, Bjorgell P, Belfrage P. Hormonal regulation of hormone-sensitive lipase in intact adipocytes: identification of phosphorylated sites and effects on the phosphorylation by lipolytic hormones and insulin. Proc Natl Acad Sci USA 1984;81:3317–21.
44 Castro Cabezas M, de Bruin TW, de Valk HW, Shoulders CC, Jansen H, Erkelens DW. Impaired fatty acid metabolism in familial combined hyperlipidemia. A mechanism associating hepatic apolipoprotein B overproduction and insulin resistance. J Clin Invest 1993;92:160–8.
45 Bredie SJH, Tack CJJ, Smits P, Stalenhoef AFH. Non-obese patients with familial combined hyperlipidemia are insulin resistant as compared with their non-affected relatives. Arterioscler Thromb Vasc Biol 1997;17:1465–71.
46 Wetterau JR, Zilversmit DB. Purification and characterization of microsomal triglyceride and cholesteryl ester transfer protein from bovine liver microsomes. Chem Phys Lipids 1985;38:205–22.
47 Wetterau JR, Zilversmit DB. A triglyceride and cholesteryl ester transfer protein associated with liver microsomes. J Biol Chem 1984;259:10863–6.
48 Wetterau JR, Aggerbeck LP, Bouma ME *et al.* Absence of microsomal triglyceride transfer protein in individuals with abetalipoproteinemia. Science 1992;258:999–1001.
49 Stalenhoef AFH, Malloy MJ, Kane JP, Havel RJ. Metabolism of apolipoproteins B-48 and B-100 of triglyceride-rich lipoproteins in patients with familial dysbetalipoproteinemia. J Clin Invest 1986;78:722–8.
50 Stalenhoef AFH, Malloy MJ, Kane JP, Havel RJ. Metabolism of apolipoproteins B-48 and B-100 of triglyceride-rich lipoproteins in normal and lipoprotein lipase-deficient humans. Proc Natl Acad Sci USA 1984;81:1839–43.
51 Hoffer MJ, Bredie SJH, Boomsma DI *et al.* The lipoprotein lipase (Asn291-Ser) mutation is associated with elevated lipid levels in familial combined hyperlipidemia. Atherosclerosis 1996;119:159–67.
52 Ito Y, Azrolan N, O'Connell A, Walsh A, Breslow JL. Hypertriglyceridemia as a result of human apo CIII gene expression in transgenic mice. Science 1990;249:790–3.
53 Wojciechowski AP, Farrall M, Cullen P *et al.* Familial combined hyperlipidaemia linked to the apolipoprotein AI-CIII-AIV gene cluster on chromosome 11q23–q24. Nature 1991;349:161–4.
54 Dallinga-Thie GM, XiangDong Bu, Van Linde-Sibenius-Trip M, Rotter JI, Lusis AJ, de Bruin TWA. Apolipoprotein A-I/C-III/A-IV gene cluster in familial combined hyperlipidemia: effects on LDL-cholesterol and apolipoproteins B and C-III. J Lipid Res 1996;37:136–47.
55 Bredie SJH, Vogelaar JM, Demacker PNM, Stalenhoef AFH. Apolipoprotein E polymorphism influences lipid phenotypic expression, but not the low density lipoprotein subfraction distribution in familial combined hyperlipidemia. Atherosclerosis 1996;126:313–24.
56 Havel RJ. The formation of LDL: mechanisms and regulation. J Lipid Res 1984;25:1570–6.
57 Deckelbaum RJ, Granot E, Oschry Y, Rose L, Eisenberg S. Plasma triglyceride determines structure-composition in low and high density lipoproteins. Arteriosclerosis 1984;4:225–31.
58 Krauss RM, Burke DJ. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. J Lipid Res 1982;23:97–105.
59 Crouse JR, Parks JS, Schey HM, Kahl FR. Studies of low density lipoprotein molecular weight in human beings with coronary artery disease. J Lipid Res 1985;26:566–74.

60 Chapman MJ, Laplaud PM, Luc G, Forgez P, Bruckert E, Goulinet S, Lagrange D. Further resolution of the low density lipoprotein spectrum in normal human plasma: physicochemical characteristics of discrete subspecies separated by density gradient ultracentrifugation. J Lipid Res 1988;29:442–58.
61 Fisher WR. Heterogeneity of plasma low density lipoproteins manifestations of the physiologic phenomenon in man. Metabolism 1983;32:283–91.
62 Swinkels DW, Hak-Lemmers HL, Demacker PNM. Single spin density gradient ultracentrifugation method for the detection and isolation of light and heavy low density lipoprotein subfractions. J Lipid Res 1987;28:1233–9.
63 Hokanson JE, Krauss RM, Albers JJ, Austin MA, Brunzell JD. LDL physical and chemical properties in familial combined hyperlipidemia. Arterioscler Thromb Vasc Biol 1995;15:452–9.
64 Dejager S, Bruckert E, Chapman MJ. Dense low density lipoprotein subspecies with diminished oxidative resistance predominate in combined hyperlipidemia. J Lipid Res 1993;34:295–308.
65 Lind BM, Littbarski R, Hohlbach G, Moller KO. Long-term investigations of serum cholesterol, serum triglyceride, and HDL cholesterol in heritable hyperlipidemic rabbits. Z Versuchstierkd 1990;33:245–9.
66 Austin MA, Brunzell JD, Fitch WL, Krauss RM. Inheritance of low density lipoprotein subclass patterns in familial combined hyperlipidemia. Arteriosclerosis 1990;10:520–30.
67 Bredie SJH, Kiemeney LA, De Haan AFJ, Demacker PNM, Stalenhoef AFH. Inherited susceptibility determines the distribution of dense low density lipoprotein subfraction profiles in familial combined hyperlipidemia. Am J Hum Genet 1996;58:812–22.
68 Slyper AH. Low-density lipoprotein density and atherosclerosis. Unraveling the connection. JAMA 1994;272:305–8.
69 Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. JAMA 1988;260:1917–21.
70 Campos H, Genest JJ, Blijlevens E *et al.* Low density lipoprotein particle size and coronary artery disease. Arterioscler Thromb 1992;12:187–95.
71 de Graaf J, Hak-Lemmers HLM, Hectors MPC, Demacker PNM, Hendriks JCM, Stalenhoef AFH. Enhanced susceptibility to *in vitro* oxidation of the dense low density lipoprotein subfraction in healthy subjects. Arterioscler Thromb 1991;11:298–306.
72 Gardner CD, Fortman SP, Krauss RM. Association of small low-density lipoprotein particles with teh incidence of coronary artery disease in men and women. JAMA 1996;276(110): 875–81.
73 Stampfer MJ, Krauss RM, Blanche PJ, Holl LG, Sacks FM, Hennekens CH. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. JAMA 1996;276(11):882–8.
74 Lamarche B, Tchernof A, Moorjani S, Cantin B, Dagenais G, Lupien PJ, Despres JP. Small dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. Circulation 1997; 95(1): 69–75.
75 Hammond MG, Fisher WR. The characterization of a discrete series of low density lipoproteins in the disease, hyper-pre-beta-lipoproteinemia. Implications relating to the structure of plasma lipoproteins. J Biol Chem 1971;246:5454–65.
76 Hammond MG, Mengel MC, Warmke GL, Fisher WR. Macromolecular dispersion of human plasma low-density lipoproteins in hyperlipoproteinemia. Metabolism 1977;26:1231–42.
77 Austin MA, Jarvik GP, Hokanson JE, Edwards K. Complex segregation analysis of LDL peak particle diameter. Genet Epidemiol 1993;10:599–604.
78 Griffin BA, Caslake MJ, Yip B, Tait GW, Packard CJ, Shepherd J. Rapid isolation of low density lipoprotein (LDL) subfractions from plasma by density gradient ultracentrifugation. Atherosclerosis 1990;83:59–67.
79 Fisher WR. The structure of the lower-density lipoproteins of human plasma: newer concepts derived from studies with the analytical ultracentrifuge. Ann Clin Lab Sci 1972;2:198–208.
80 de Graaf J, Swinkels DW, De Haan AFJ, Demacker PNM, Stalenhoef AFH. Both inherited susceptibility and environmental exposure determine the low density lipoprotein subfraction pattern distribution in healthy Dutch families. Am J Hum Genet 1992;51:1295–310.
81 Dormans TP, Swinkels DW, de Graaf J, Hendriks JCM, Stalenhoef AFH, Demacker PNM. Single-spin density-gradient ultracentrifugation vs. gradient gel electrophoresis: two methods for detecting low-density-lipoprotein heterogeneity compared. Clin Chem 1991;37:853–8.
82 Demacker PNM, Otvos JD, Schmitz G *et al.* Alternative approaches to lipoprotein analysis. In: Laboratory measurement of lipids, lipoproteins and apolipoproteins, Rifar N, Warnick GR, eds. Washington: AACC Press, 1994: 323–347.
83 Marzetta CA, Foster DM, Brunzell JD. Conversion of plasma VLDL and IDL precursors into various LDL subpopulations using density gradient ultracentrifugation. J Lipid Res 1990;31:975–84.
84 Teng B, Sniderman AD, Soutar AK, Thompson GR. Metabolic basis of hyperapobetalipoproteinemia. Turnover of apolipoprotein B in low density lipoprotein and its precursors and subfractions compared with normal and familial hypercholesterolemia. J Clin Invest 1986;77:663–72.
85 Fisher WR, Zech LA, Bardalaye P, Warmke G, Berman M. The metabolism of apolipoprotein B in subjects with hypertriglyceridemia and polydisperse LDL. J Lipid Res 1980;21:760–74.
86 Ginsberg HN, Ngai C, Wang XJ, Ramakrishnan R. Increased production rates of LDL are common in individuals with low plasma levels of HDL cholesterol, independent of plasma triglyceride concentrations. Arterioscler Thromb 1993;13:842–51.
87 Deckelbaum RJ, Eisenberg S, Oschry Y, Butbul E, Sharon I, Olivecrona T. Reversible modification of human plasma low density lipoproteins toward triglyceride-rich precursors. A mechanism for losing excess cholesterol esters. J Biol Chem 1982;257:6509–17.
88 Granot E, Deckelbaum RJ, Eisenberg S, Oschry Y, Bengtsson-Olivecrona G. Core modification of human low-density lipoprotein by artificial triacylglycerol emulsion. Biochim Biophys Acta 1985;833:308–15.
89 Gambert P, Bouzerand-Gambert C, Athias A, Farnier M, Lallemant C. Human low density lipoprotein subfractions separated by gradient gel electrophoresis: composition, distribution, and alterations induced by cholesteryl ester transfer protein. J Lipid Res 1990;31:1199–210.
90 Levy E, Deckelbaum RJ, Thibault RL, Seidman E, Olivecrona T, Roy CC. In vitro remodelling of plasma lipoproteins in whole plasma by lipoprotein lipase in primary and secondary hypertriglyceridaemia. Eur J Clin Invest 1990;20:422–31.
91 Griffin BA, Packard CJ. Metabolism of VLDL and LDL subclasses. Curr Opin Lipidol 1994;5:200–6.
92 Watson TD, Caslake MJ, Freeman DJ *et al.* Determinants of LDL subfraction distribution and concentrations in young normolipidemic subjects. Arterioscler Thromb 1994;14:902–10.
93 Zambon A, Austin MA, Brown BG, Hokanson JE, Brunzell JD. Effect of hepatic lipase on LDL in normal men and those with coronary artery disease. Arterioscler Thromb 1993;13:147–53.
94 Krauss RM. Heterogeneity of plasma low-density lipoproteins and atherosclerosis risk. Curr Opin Lipidol 1994;5:339–49.
95 Packard CJ, Munro A, Lorimer AR, Gotto AM, Shepherd J. Metabolism of apolipoprotein B in large triglyceride-rich very low density lipoproteins of normal and hypertriglyceridemic subjects. J Clin Invest 1984;74:2178–92.
96 Krauss RM, Hellerstein MK, Neese RA, Blanche PJ, LaBelle MA, Shames DM. Altered metabolism of large very low density lipoproteins in subjects with a predominance of small low dense lipoproteins (abstract). Circulation 1995;92(8):I–102(0480).
97 Austin MA. Genetics of low-density lipoprotein subclasses. Curr Opin Lipidol 1993;4:125–32.
98 Austin MA, King MC, Vranizan KM, Newman B, Krauss RM. Inheritance of low-density lipoprotein subclass patterns: results of complex segregation analysis. Am J Hum Genet 1988;43:838–46.
99 Fisher WR, Hammond MG, Mengel MC, Warmke GL. A genetic determinant of the phenotypic variance of the molecular weight of low density lipoprotein. Proc Natl Acad Sci USA 1975;72:2347–51.

100 Austin MA, Newman B, Selby JV, Edwards K, Mayer EJ, Krauss RM. Genetics of LDL subclass phenotypes in women twins. Concordance, heritability, and commingling analysis. Arterioscler Thromb 1993;13:687–95.

101 Nishina PM, Johnson JP, Naggert JK, Krauss RM. Linkage of atherogenic lipoprotein phenotype to the low density lipoprotein receptor locus on the short arm of chromosome 19. Proc Natl Acad Sci USA 1992;89:708–12.

102 Haffner SM, Stern MP, Miettinen H, Robbins D, Howard BV. Apolipoprotein E polymorphism and LDL size in a biethnic population. Arterioscler Thromb Vasc Biol 1996;16(9):1184–1188.

103 Vohl M-C, Tchernof A, Dionne FT *et al.* The apoB-100 gene *Eco*RI polymorphism influences the relationship between features of the insulin resistance syndrome and the hyper-apoB and dense LDL phenotype in men. Diabetes 1996;45:1405–11.

104 Austin MA, Wijsman E, Guo SW, Krauss RM, Brunzell JD, Deeb S. Lack of evidence for linkage between low-density lipoprotein subclass phenotypes and the apolipoprotein B locus in familial combined hyperlipidemia. Genet Epidemiol 1991;8:287–97.

105 Rotter JI, Bu X, Cantor RM *et al.* Multilocus genetic determinants of LDL particle size in coronary artery disease families. Am J Hum Genet 1996;58:585–94.

106 Swinkels DW, Hendriks JCM, Demacker PNM, Stalenhoef AFH. Differences in metabolism of three low density lipoprotein subfractions in Hep G2 cells. Biochim Biophys Acta 1990;1047:212–22.

107 de Graaf J, Hendriks JCM, Swinkels DW, Demacker PNM, Stalenhoef AFH. Differences in LDL receptor-mediated metabolism of three low density lipoprotein subfractions by human monocyte-derived macrophages: impact on the risk for atherosclerosis. Artery 1993;20:201–30.

108 Nigon F, Lesnik P, Rouis M, Chapman MJ. Discrete subspecies of human low density lipoproteins are heterogeneous in their interaction with the cellular LDL receptor. J Lipid Res 1991;32:1741–53.

109 Lagrost L, Gandjini H, Athias A, Guyard-Dangremont V, Lallemant C, Gambert P. Influence of plasma cholesteryl ester transfer activity on the LDL and HDL distribution profiles in normolipidemic subjects. Arterioscler Thromb 1993;13:815–25.

110 Tribble DL, Holl LG, Wood PD, Krauss RM. Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. Atherosclerosis 1992;93:189–99.

111 Swinkels DW, Demacker PNM, Hendriks JCM, van 't Laar A. Low density lipoprotein subfractions and relationship to other risk factors for coronary artery disease in healthy individuals. Arteriosclerosis 1989;9:604–13.

112 Goldstein JL, Ho YK, Basu SK, Brown MS. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. Proc Natl Acad Sci USA 1979;76:333–7.

113 Ross R. The pathogenesis of atherosclerosis – an update. N Engl J Med 1986;314:488–500.

114 Goldstein JL, Brown MS. The low-density lipoprotein pathway and its relation to atherosclerosis. Annu Rev Biochem 1977;46:897–930.

115 Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989;320:915–24.

116 Quinn MT, Parthasarathy S, Fong LG, Steinberg D. Oxidatively modified low density lipoproteins: a potential role in recruitment and retention of monocyte/macrophages during atherogenesis. Proc Natl Acad Sci USA 1987;84:2995–8.

117 Henriksen T, Mahoney EM, Steinberg D. Enhanced macrophage degradation of low density lipoprotein previously incubated with cultured endothelial cells: recognition by receptors for acetylated low density lipoproteins. Proc Natl Acad Sci USA 1981;78:6499–503.

118 Steinbrecher UP, Zhang HF, Lougheed M. Role of oxidatively modified LDL in atherosclerosis. Free Radic Biol Med 1990;9:155–68.

119 Steinberg D. The oxidative modification hypothesis of atherogenesis: strengths and weaknesses. In: Woodford FP, Davignon J, Sniderman A, eds. Atherosclerosis X, International congress series 1066, New York: Elsevier, 1995:25–9.

120 Sparrow CP, Parthasarathy S, Steinberg D. A macrophage receptor that recognizes oxidized low density lipoprotein but not acetylated low density lipoprotein. J Biol Chem 1989;264:2599–604.

121 Endemann G, Stanton LW, Madden KS, Bryant CM, White RT, Protter AA. CD36 is a receptor for oxidized low density lipoprotein. J Biol Chem 1993;268:11811–16.

122 de Rijke YB, van Berkel TJ. Rat liver Kupffer and endothelial cells express different binding proteins for modified low density lipoproteins. Kupffer cells express a 95-kDa membrane protein as a specific binding site for oxidized low density lipoproteins. J Biol Chem 1994;269:824–7.

123 Ottnad E, Parthasarathy S, Sambrano GR *et al.* A macrophage receptor for oxidized low density lipoprotein distinct from the receptor for acetyl low density lipoprotein: partial purification and role in recognition of oxidatively damaged cells. Proc Natl Acad Sci USA 1995;92:1391–5.

124 Chait A, Brazg RL, Tribble DL, Krauss RM. Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. Am J Med 1993;94:350–6.

125 Tribble DL, van den Berg JJ, Motchnik PA *et al.* Oxidative susceptibility of low density lipoprotein subfractions is related to their ubiquinol-10 and alpha-tocopherol content. Proc Natl Acad Sci USA 1994;91:1183–7.

126 de Rijke YB, Bredie SJH, Demacker PNM, Vogelaar JM, Hak-Lemmers HLM, Stalenhoef AFH. The redox status of coenzym Q10 in total LDL as an indicator of *in vivo* oxidative modification; studies in subjects with familial combined hyperlipidemia. Arterioscler Thromb Vasc Biol 1997;17:127–33.

127 Grundy SM. HMG-CoA reductase inhibitors for treatment of hypercholesterolemia. N Engl J Med 1988;319:24–33.

128 Franceschini G, Cassinotti M, Vecchio G *et al.* Pravastatin effectively lowers LDL cholesterol in familial combined hyperlipidemia without changing LDL subclass pattern. Arterioscler Thromb 1994;14:1569–75.

129 de Graaf J, Demacker PNM, Stalenhoef AFH. The effect of simvastatin treatment on the low-density lipoprotein subfraction profile and composition in familial hypercholesterolaemia. Neth J Med 1993;43:254–61.

130 Grundy SM, Vega GL. Fibric acids: effects on lipids and lipoprotein metabolism. Am J Med 1987;83:9–20.

131 de Graaf J, Hendriks JCM, Demacker PNM, Stalenhoef AFH. Identification of multiple dense LDL subfractions with enhanced susceptibility to *in vitro* oxidation among hypertriglyceridemic subjects. Normalization after clofibrate treatment. Arterioscler Thromb 1993;13:712–19.

132 Hokanson JE, Austin MA, Zambon A, Brunzell JD. Plasma triglyceride and LDL heterogeneity in familial combined hyperlipidemia. Arterioscler Thromb 1993;13:427–34.

133 Cortner JA, Coates PM, Liacouras CA, Jarvik GP. Familial combined hyperlipidemia in children: clinical expression, metabolic defects, and management. J Pediatr 1993;123:177–84.

134 Schaefer EJ, Levy RI. Pathogenesis and management of lipoprotein disorders. N Engl J Med 1985;312:1300–10.

135 Rose HG, Haft GK, Juliano J. Clofibrate-induced low density liporotein elevation. Therapeutic implications and treatment by colestipol resin. Atherosclerosis 1976;23:413–27.

136 Angelin B, Hershon KS, Brunzell JD. Bile acid metabolism in hereditary forms of hypertriglyceridemia: evidence for an increased synthesis rate in monogenic familial hypertriglyceridemia. Proc Natl Acad Sci USA 1987;84:5434–8.

137 Esterbauer H, Striegl G, Puhl H, Oberreither S, Rotheneder M, el-Saadani M, Jurgens G. The role of vitamin E and carotenoids in preventing oxidation of low density lipoproteins. Ann NY Acad Sci 1989;570:254–67.

138 Reaven PD, Khouw A, Beltz WF, Parthasarathy S, Witztum JL. Effect of dietary antioxidant combinations in humans. Protection

of LDL by vitamin E but not by beta-carotene. Arterioscler Thromb 1993;13:590–600.
139 Parthasarathy S, Young SG, Witztum JL, Pittman RC, Steinberg D. Probucol inhibits oxidative modification of low density lipoprotein. J Clin Invest 1986;77:641–4.
140 Kleinveld HA, Naber AHJ, Stalenhoef AFH, Demacker PNM. Oxidation resistance, oxidation rate, and extent of oxidation of human low-density lipoprotein depend on the ratio of oleic acid content to linoleic acid content: studies in vitamin E deficient subjects. Free Radic Biol Med 1993;15:273–80.
141 Stampfer MJ, Hennekens CH, Manson JE, Colditz GA, Rosner B, Willett WC. Vitamin E consumption and the risk of coronary disease in women [see comments]. N Engl J Med 1993;328:1444–9.
142 Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. N Engl J Med 1993;328:1450–6.
143 Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 1993;342:1007–11.
144 The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 1994;330:1029–35.
145 Kushi LH, Folksom AR, Prineas RJ, Mink PJ, Wu Y, Bostick RM. Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women. N Engl J Med 1996;334:1156–62.
146 Randomized trial of vitamin E in patients with coronary diesease: Cambridge Heart Antioxidant Study (CHAOS). Lancet 1996;347:781–86.
147 Nawrocki JW, Weiss SR, Davidson MH *et al.* Reduction of LDL cholesterol by 25% to 60% in patients with primary hypercholesterolemia by atorvastatin, a new HMG-CoA reductase inhibitor. Arterioscler Thromb Vasc Biol 1995;15:678–82.
148 Bakker-Arkema RC, Davidson MH, Goldstein RJ *et al.* Efficacy and safety of a new HMG-CoA reductase inhibitor, atorvastatin, in patients with hypertriglyceridemia. JAMA 1996;275:128–133.